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Analysis of chromatin, nuclear DNA and organelle composition in somatic hybrids between *Solanum tuberosum* and *Solanum sanctae-rosae*

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Abstract A protoplast fusion strategy has been applied to advance aspects of a potato breeding programme. A sub-population of somatic hybrids, selected for agronomic potential, between tetraploid *Solanum tuberosum* cv. Brodick and a diploid EBN2 accession, *S. sanctae-rosae* was subjected to detailed molecular analysis. This study reports the use of simple sequence repeats (SSRs) to identify nuclear hybrid genomes and PCR and DNA-DNA analysis to determine organelle composition in somatic hybrids derived from these parents. SSR analysis revealed somatic hybrids containing the genetic background of *S. tuberosum* cv. Brodick with some specific markers from *S. sanctae-rosae*. One somatic hybrid contained the chloroplasts derived from *S. sanctae-rosae*, and several hybrids had detectable RFLP mitochrondrial DNA profiles, indicating genetic re-arrangements. We also examined the use of DNase I sensitivity to the genomic and ribosomal RNA sequences in these somatic hybrids as an indicator of changes in chromatin structure. Chromatin and DNAse I analysis showed differential sensitivity to increasing levels of nuclease; DNA from several somatic hybrids was found to be resistant to DNase I compared to the parental plants. The significance of the findings to somatic cell genetics and plant breeding studies is discussed.

Key words *Solanum* somatic hybrids · Organelle · Nuclear and chromatin analysis

Introduction

Plant protoplasts have been useful in providing an alternative means of producing hybrids from sexually incompatible potato species (Carputo et al. 1995). This technique has the potential for self-generating biodiversity in the numerous nuclear and cytoplasmic hybrid combinations along with possible recombination events between parental genomes (Kumar and Cocking 1987). There have been numerous reports on the production and analysis of *Solanum* somatic hybrids (Novy and Helgeson 1994); certain somatic products express various traits; including disease resistance to viruses (Thach et al. 1993; Pehu et al. 1990), bacteria (Austin et al. 1988), fungi (Cooper-Bland et al. 1996; Mattheij et al. 1992) and insect pests (Austin et al. 1993; Cooper-Bland et al. 1994).

An analysis of putative fusion products is essential to confirm hybrid status (Masuelli et al. 1995; Penner et al. 1996; Matthews et al. 1997) and expression of the desirable trait(s). This can be done via morphological, biochemical, cytological and molecular markers (Harding 1996; Pinto et al. 1995), and increasingly sophisticated molecular discrimination methods have recently been reported (Provan et al. 1996; Matthews et al. 1999). Gene products from the nuclear and organelle genomes are known to interact within cells (Hanson and Conde 1986), resulting in functional proteins for essential metabolism (ATP generation and photosynthesis). An understanding of the parental nuclear components and organelle composition of these hybrids would be of interest in cytoplasmic genetics and agronomic studies in potato breeding (Lossl et al. 1994). The characterisation of mitochondrial DNA (mtDNA) and chloroplast DNA (cpDNA) in several *Solanum* hybrids has been reported (Pehu et al. 1989; Xu 1993; Xu et al. 1993). Restriction enzyme fragment length polymorphisms (RFLPs) have also been informative indicators of recombination in mtDNA (Kemble et al. 1986), and the use of specific mtDNA probes has shown genetic change in potato somatic hybrids (Xu et al. 1993).

Unlike conventional plant breeding, where fertilisation results in seed formation, the resultant heterokaryon from protoplast fusion develops into a plantlet. In the heterokaryon, interacting parental cytoplasms segregate where one set of organelles predominant; presumably

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this nuclear/cytoplasmic combination is stable and confers some selective advantage. There are interacting genetic components (symeteric/organelle combinations) likely to influence plantlet vigour, resulting in active growth and development (heterosis). There is also evidence for the genetic recombination and rearrangement of organelle DNA in some somatic hybrids (Kanno et al. 1997). This is less so with cpDNA than with mtDNA, where recombination sequences have been identified in several plant mitochondrial genomes (Stern and Palmer 1984). Heterokaryons are likely to exchange genetic information, inter-phase nuclei are certain to interact, and the correct function of regulatory mechanisms are essential for active gene expression.

Chromatin conformation and nucleosomal structure have been implicated as a cellular processes that undergo modulation during genetic modification (van Blokland et al. 1997; Kumpatla et al. 1998; Cheng et al. 1998; Kadonaga et al. 1998). As chromatin relaxes, DNA no longer becomes associated with histones in nucleosome structures and is vulnerable to enzymic digestion. This is a characteristic of chromatin and its relationship to gene expression (Workman and Kingston 1998). In this paper, we report the use of simple-sequence repeats (SSRs) to identify nuclear hybrid genomes, and the polymerase chain reaction (PCR) and DNA-DNA analysis to determine organelle composition in somatic hybrids derived from *Solanum sanctae-rosae* and *S. tuberosum*; we also examine DNase I sensitivity to the ribosomal RNA sequences in these somatic hybrids.

Materials and methods

Plant material

Solanum germplasm was obtained from the Commonwealth Potato Collection (CPC), Scottish Crop Research Institute: *S. sanctae-rosae* (CPC 3779, Sct) and *S. tuberosum* cv. Brodick (Brod). Somatic hybrids were produced from a symmetric protoplast fusion experiment using *S. sanctae-rosae* and Brod as parents as described by Harding and Millam (1999) and maintained in tuber form. This material was selected for its resistance to potato cyst nematode (PCN) and maintained in tuber form for several generations (under field conditions) for assessments of phenotypic stability.

Preparation of total DNA and organelle DNA

Plants derived from some 65 *S. sanctae-rosae* and *S. tuberosum* cv Brodick putative somatic hybrids were assessed. Total DNA was isolated from selected leaf material as described by Harding and Benson (1995). Chloroplasts were prepared from fresh leaves, and DNA (cpDNA) was isolated using a modification of the Kumar and Cocking (1982) procedure without CsCl-EtBr equilibrium density gradient centrifugation. Briefly, leaves were liquidised in chilled homogenised buffer and the homogenate centrifuged at low speed (500 *g*) for 10 min to remove nuclei/starch. The supernatant was centrifuged at 3,000 *g* for 10 min to isolate the chloroplasts; the resulting pellet was suspended in homogenisation buffer, then loaded onto a 30–60% sucrose gradient and centrifuged in a swing-out rotor (SW40.1 Beckman) for 45 min at 20,000 rpm. Chloroplasts were removed from the gradient, lysated in 2% sarkosyl and cpDNA was extracted as described (Kumar and Cocking 1982). Similarly, mitochrondria were isolated and DNA (mtDNA) prepared according to the procedure described by Bland et al. (1985). Tubers of somatic hybrids were homogenised in chilled buffer and differentially centrifuged to remove starch, nuclei and chloroplasts. The supernatant was centrifuged at 11,000 rpm (Sorval, GSA rotor) for 20 min; the resulting mitochrondrial pellet was treated with DNase I, washed before loading onto a 1.2–1.6 *M* sucrose step gradient and centrifuged in a swing-out rotor (SW40.1 Beckman) for 60 min at 25,000 rpm. Mitochrondria were washed, lysated and the mt-DNA phenol/chloroform extracted as described by Bland et al. (1985).

Restriction enzyme digestion, gel electrophoresis and Southern blotting

Isolated organelle DNA was digested with several restriction enzymes, and the DNA fragments were fractionated by agarose gel electrophoresis and transferred to nylon membrane (Tropix) by Southern blotting in 20 × SSC as described by Harding et al. (1996).

DNA hybridisation and detection of sequences by chemiluminescence

Organelle DNA was biotin-labelled by the method of nick-translation and hybridised to nylon membranes overnight as described by Harding and Benson (1995).

SSR analysis

The PCR reaction, amplification and electrophoresis of PCR products were essentially performed as described by Charters et al. (1996). Briefly, PCR reactions contained 1 U *Taq* DNA polymerase, 1.5 m*M* MgCl2 buffer, 0.2 m*M* dNTPs, 0.3 μ*M* of a single primer (the microsatellite 88, BDB-[CA]7) and 20 ng of potato DNA. PCR amplifications were performed on a Hybaid Omnigene Thermocycler using the following programme: 30 cycles of 1 min at 94°C, 2 min at 55°C, and 30 s at 72°C and a final 5-min extension at 72°C. Loading buffer (5 μl, 5 *M* urea, 0.02% bromophenol blue) was added to each final reaction. Resolution of PCR products was done on a Multiphor II flat-bed system (Pharmacia Biotech) with pre-cast polyacrylamide gels (Cleangel 48 S, Pharmacia Biotech). Electrophoresis was performed in 0.2 *M* Tris base, 0.2 *M* tricine, 0.55% (w/v) SDS in a three-stage programme: (1) 20 min at 200 V, 20 mA, 10 W; (2) 50 min at 380 V, 30 mA, 20 W (3) 30 min at 450 V, 30 mA, and 20 W. Detection of PCR products was done with a plusone DNA silver staining kit (Pharmacia Biotech).

PCR amplification of cpDNA and mtDNA

The PCR reactions were performed using 10–50 ng of total DNA with the tobacco cpSSR primers described by Provan (1998). NTCP9 specifies the *trn*G/*trn*R intergenic region of the sequence 5'-CTTCCAAGCTAACGATGC-3' and 5'-CTGTCCTATCCATTAGACAATG-3'. PCR products of 300 bp were generated using the amplification programme: (1) 94°C, 3 min, 1 cycle; (2) 94°C, 15 s; 55°C, 30 s; 72°C, 30 s, 35 cycles, with a final 5-min extension at 72°C. PCR products were detected by staining the 2% agarose gel after electrophoresis with ethidium bromide. The mitochrondrial universal primers for the *nad*1 exon B/C gene, 5'-GCATTACGATCTGCAGCTCA-3' and 5'-GGAGCTCGATTAGTTTCTGC-3' (Demesure et al. 1995), were used in a PCR amplication cycle (1) 94°C, 60 s; 37°C, 120 s; 72°C, 120 s, 40 cycles, with a 5-min extension at 72°C. PCR products were fractionated by gel electrophoresis as described above.

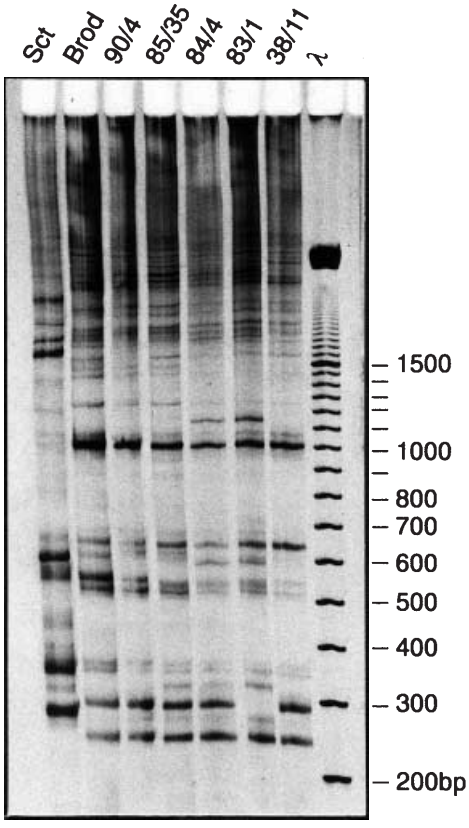

Fig. 1 Simple sequence repeat (SSR) analysis of DNA derived from *S. sanctae-rosae* (*Sct, lane 1*), *S. tuberosum* cv. Brodick (*Brod, lane 2*) and somatic hybrids (*lanes 3–7*)

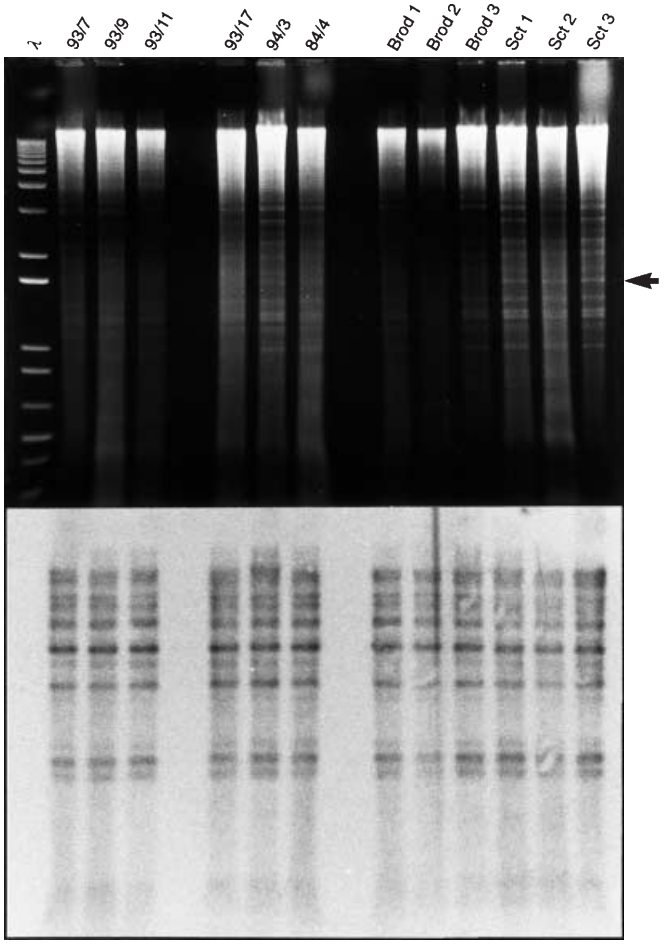

Fig. 2 Top An ethidium bromide-stained gel containing total DNA, digested with *Bam*HI, from *S. sanctae-rosae* with *S. tuberosum* cv. Brodick and somatic hybrids. **Bottom** Same gel Southern-blotted, and hybridised to biotin-labelled cpDNA and the hybridised cpDNA sequences detected by chemiluminescence (bottom). The molecular-weight marker represents fragments 0.1, 0.2, 0.4, 0.6, 0.8, 1.0, 1.7, 2.0, 3.0, 4.0, 5.0 to 15 kb (from gel bottom to top)

Preparation of chromatin and DNAse I assay

Leaves were prepared, homogenised and nuclei prepared and assayed with DNAse I as described previously (Paul and Ferl 1988). Essentially, nuclei were dispensed in 500-µl aliquots. A DNase I dilution series was prepared (0, 10, 20, 40, 80 and 200 µg ml-1) where 10 µl of each dilution was added to each nuclei preparation. These were incubated at 37°C for 1 min then 30°C for 9 min. Nuclei were pelleted for 10 s at 10,000 *g* (Microfuge), then suspended in 300 µl of cold (4°C) extraction buffer (0.1 *M* TRIS.HCl, pH 8.0; 50 m*M* EDTA; 0.5 *M* NaCl and 10 m*M* beta-mercaptoethanol) with 5 µl 20 mg ml-1 proteinase K and 50 µl 10% sodium dodecyl sulfate. Incubation was at 65°C for 45–60 min, after which 100 µl of 5 *M* potassium acetate was added and the mixture held on ice for 30 min. The mixtures were centrifuged for 10 min at 10,000 *g* and the supernatant transferred to eppendorfs; 600 µl of isopropanol was added and the solution stored at –20°C for 1 h. DNA was centrifuged for 10 min at 10,000 *g*, the supernatant drained off and the pellet suspended in 25–50 µl of TE (10 m*M* TRIS.HCl, 1 m*M* EDTA, pH 8.0) buffer. These samples were fractionated by gel electrophoresis, Southern blotted, hybridised to an rDNA probe pTa 71 (Gerlach and Bedbrook 1979) and the sequences detected by chemiluminescence as described above.

Results

Plantlets regenerating from protoplast fusion experiments are likely to give rise to a combination of symmetric plants, asymmetric hybrids, numerous nuclear and cytoplasmic combinations and some plantlets of parental origin. It is important to distinguish these events. The use of simple-sequence repeats (SSRs) to identify nuclear hybrid genomes, PCR and DNA-DNA analysis to determine the organelle composition in somatic hybrids derived from *S. sanctae-rosae* and *S. tuberosum* and DNase I sensitivity to the ribosomal RNA sequences in these somatic hybrids were evaluated in the investigation presented here.

Figure 1 shows the SSR analysis of several somatic hybrids derived from *S. sanctae-rosae* with *S. tuberosum* cv. Brodick. The DNA profiles show that there are several main fragments in the molecular weight range 250, 300, 525, 650 and 1000 bp in the hybrids similar to *S. tuberosum* cv. Brodick. Higher molecular weight fragments, those greater than 1,500 bp, were all characteristic of *S. tuberosum* cv. Brodick, while only a few fragments, 275 and 325 bp corresponded to *S. sanctae-rosae*. Notably, some main Brodick fragments, 300 bp and 600 bp,

were absent in somatic hybrids 83/1 and 38/11, respectively, suggestive of possible deletion events or chromosomal elimination.

Figure 2 (top) shows DNA fragments in an ethidium bromide stained gel containing total DNA digested with *BamHI* from *S. sanctae-rosae*, *S. tuberosum* cv. Brodick and several somatic hybrids. The main restriction enzyme fragments are derived from both repetitive genomic DNA and cpDNA. These DNA profiles are similar in all digests, however, in the digestions of *S. sanctae-rosae* (mid-gel) there is a single unique fragment (see arrow) which is absent in the *S. tuberosum* cv. Brodick digests. This unique restriction fragment is present in hybrids 84/4 and 94/3. This gel was then Southern blotted and hybridised to biotin-labelled cpDNA, and the hybridised cpDNA sequences were detected by chemiluminescence (bottom). All cpDNA hybridised fragments were identical between the parents and hybrids. This demonstrated the stability of the chloroplast genomes. These fragments did not correspond to those unique DNA fragments of *S. sanctae-rosae* found in the ethidium bromide-stained gel, indicating that the unique *Bam*HI fragment was nuclear DNA.

Some 6 primer pairs of the tobacco cpSSR described by Provan (1998) were tested for PCR product differences. (Only NTCP9 specifying the *trn*G/*trn*R intergenic region gave a product of approximately 300 bp.) Figure 3 shows an ethidium bromide stained gel containing cpDNA PCR-amplified products. Total DNA from some 25 somatic hybrids were PCR-analysed; most contained chloroplasts derived from *S. tuberosum* cv. Brodick, except for hybrids 94/3, which had chloroplasts from *S. sanctae-rosae*. The molecular-weight difference in the PCR products between *S. tuberosum* cv. Brodick and *S. sanctae-rosae* was approximately 25 bp. Without screening the entire chloroplast genome with restriction enzymes (see Fig. 2), it would be highly unlikely to detect polymorphic differences by conventional restriction fragment length polymorphic (RFLP) analysis.

PCR analysis with several universal primers (Demesure et al. 1995) did not reveal detectable differences in PCR products between *S. tuberosum* cv. Brodick and *S. sanctae-rosae*, whereas, RFLPs were recognised in several mtDNA profiles after digestion with *Bam*HI, *Bgl*II, *Eco*RI and *Sma*I. Figure 4 shows an ethidium bromide-stained gel containing mtDNA fragments after digestion with *Bam*HI, *Eco*RI and *Sma*I. An RFLP can be seen to be present in several somatic hybrid digests (see arrow, left side, mid gel) of *Bam*HI relative to Brod and the somatic hybrids 38/11, 49/4, 84/6 and 83/1 (right side, mid gel). Also in hybrid 83/1 (see arrow, right side), a *Bam*HI fragment is missing compared to hybrids 84/6, 49/4, 38/11 and Brod. Similar observations can be made in mtDNA profiles after digestion with *Eco*RI, with missing fragments in hybrids 90/4 and 90/10 (left side) and 83/4 (right side), and several missing *Sma*I fragments in Brod and hybrid 90/10 (right side). These differing mtDNA profiles in the hybrids possibly indicate genetic re-arrangements within the mitochrondrial genome. Despite these observations, the somatic hybrids have a

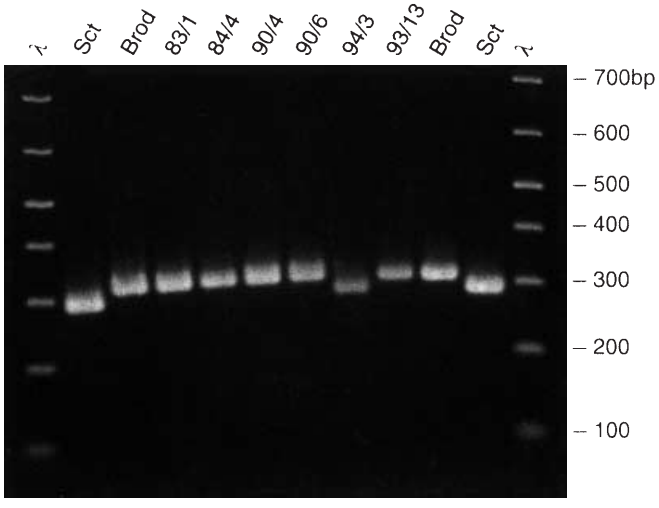

Fig. 3 An ethidium bromide-stained gel containing cpDNA PCR-amplified products

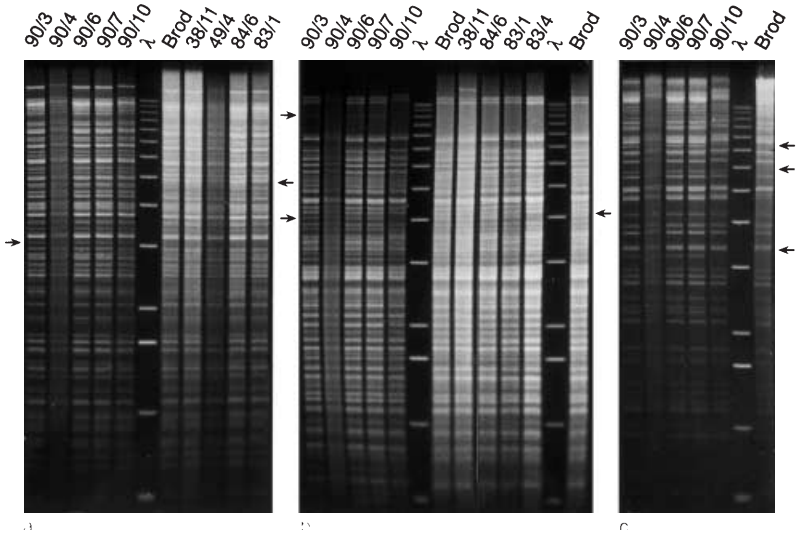

Fig. 4a–c An ethidium bromide-stained gel containing mtDNA restriction enzyme fragments after digestion with *Bam*HI (**a**), *Eco*RI (**b**) and *Sma*I (**c**). The synthetic marker is a ladder of the following fragments: 15, 12, 10, 9, 8, 7, 6, 5, 4, 3, 2, 1.7, 1, 0.8 kb (from top to bottom)

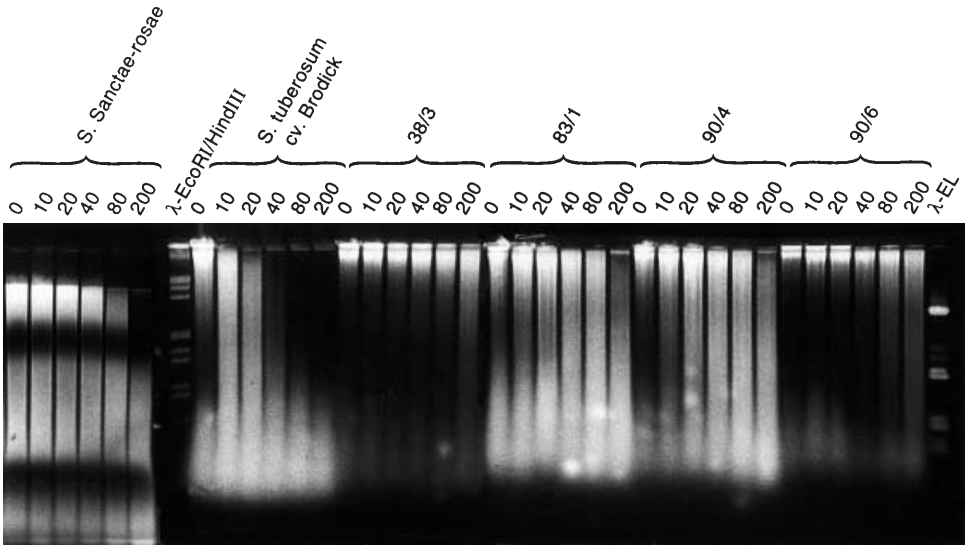

Fig. 5 An ethidium bromide stained gel containing nuclei derived from *S. tuberosum* cv. Brodick, *S. sanctae-rosae* and the somatic hybrids sequentially digested with increasing amounts of DNase I. Lambda *Eco*RI/*Hind*III markers represent (gel top) 21.1, 5.1/5.0, 4.3, 3.5, 2.0, 1.9, 1.6, 1.4, 0.95, 0.83 and 0.56 kb (gel bottom) and the lambda EL marker is a partial digest of lambda DNA end-labelled with biotin (fragment sizes include: 21.1, 5.0, 4.3, 3.5, 2.0, 1.9, 1.6, 1.4, 0.8 and 0.6 kb)

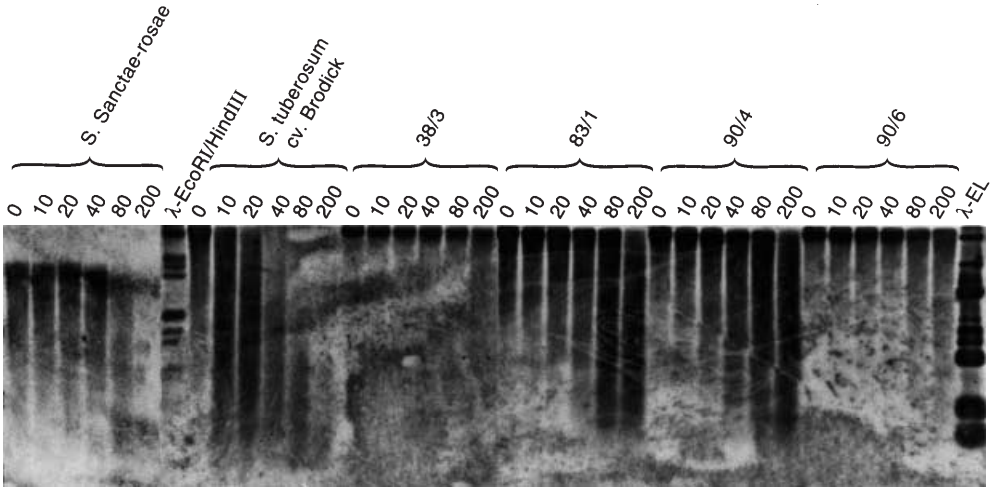

Fig. 6 Luminograph of rDNA profiles of nuclei derived from *S. tuberosum* cv. Brodick, *S. sanctae-rosae* and the somatic hybrids sequentially digested with increasing amounts of DNase I following Southern blotting and hybridisation to the rDNA probe

DNA profile showing the inheritance of mitochrondria from *S. tuberosum* cv. Brodick.

Nuclei were isolated from *S. sanctae-rosae*, *S. tuberosum* cv. Brodick and their somatic hybrids and tested for their sensitivity to DNase I. Figure 5 shows an ethidium bromide-stained gel containing nuclei from these plants sequentially digested with increasing amounts of DNase I. Nuclei derived from *S. tuberosum* cv. Brodick were effectively degraded with 20 µg ml-1 DNase I, *S. sanctae-rosae* nuclei were substantially degraded with 40 µg ml-1 DNaseI, whereas hybrids 83/1 and 90/4 were relatively resistant up to 80 µg ml-1 DNase I and hybrids 38/3 and 90/6 showed resistance up to 200 µg ml-1 DNase I.

These gels were Southern-blotted and hybridised to the rDNA probe pTa 71 (Fig. 6). The size marker was as Fig. 5. Similar patterns of sensitivity to DNase I were seen in the ribosomal RNA gene of the NOR region in *S. tuberosum* cv. Brodick, *S. sanctae-rosae* and their somatic hybrids compared to their genomic samples (Fig. 5). rDNA profiles for S.tuberosum cv. Brodick were degraded after 40 µg ml-1 of DNase I, whereas *S. sanctae-rosae* was degraded after 80 µg ml-1 of DNase I. Degradation of the rDNA gene sequences for hybrids 83/1 and 90/4 showed greater levels of resistance than the DNA derived from the parental nuclei. Surprisingly, the rDNA nucleosomal arrays of hybrids 38/3 and 90/6 were found to be resistant to 200 µg ml-1. The evidence in Figs. 5 and 6 suggests there are differences in the nucleosome structures and chromatin organisation between the parents and hybrids. The higher levels of resistance to DNase I indicate tight binding of histone proteins to DNA strands and shortened inter-nucleosome distances (<200 bp). These findings suggest that the alterations in chromatin organisation are commensurate with regions of transcriptional inactivity in genome and rDNA sequences.

Discussion

Potato breeding is an example of a crop in which the use of alternative techniques to exchange and introgress 'new' genetic information conferring beneficial traits from wild species into conventional cultivars offers advantages (Millam et al. 1997). Tissue culture procedures to produce somatic hybrids and the application of molecular biological tools for their analysis are well-established. Fundamental to the characterisation of this 'new germplasm' is an understanding of nuclear components, organelle segregation patterns and possible factors affecting gene expression. A previous analysis of somatic hybrids derived from the parents S.*tuberosum* cv. Brodick and *S. sanctae-rosae* detected differences in ribosomal RNA gene profiles suggestive of genetic recombination between the NOR regions of chromosome 2 (Harding and Millam 1999). Also, considerable changes in rDNA methylation were found in these somatic hybrids compared to the parents. The present study was conducted to extended to further examine the genetic/molecular background of these somatic hybrids.

Analysis by simple-sequence repeats has been readily applied to *Brassica* species (Charters et al. 1996) and is an effective technique to screen somatic hybrids of *S. tuberosum* (Provan et al. 1996). Its application is shown in Fig. 1. Analysis of the somatic hybrids showed DNA profiles with several fragments similar to *S.tuberosum* cv. Brodick and only a few fragments corresponding to *S. sanctae-rosae*. This is useful in confirming the genetic background of Brodick as an objective in the accelerated breeding programme (Millam et al. 1997). Notwithstanding the limited application of SSR analysis, apart from 'marking bands on a gel' to confirm hybridity, it requires further development to identify chromosomal specific markers in novel germplasm to truly aid marker-assisted selection in breeding programmes (Stadler et al. 1995; Zanke and Hemleben 1997).

Protoplast fusion techniques result in the formation of heterokaryons. One of the principal disadvantages of the somatic hybrid approach, indeed one of the main reasons precluding its more widespread uptake and application, is that the resultant hybrid will be a random mixture of the two parental genomes. For the generation of material used in this study derived from a symmetric protoplast fusion experiment, the initial selection was based on hybrid vigour (heterosis) of the regenerating material (Barr 1996). In the absence of a selection system, the cellular composition of these hybrids is likely to be entirely random (Waara and Glimelius 1995) with only tissue factors affecting the development of these plants (Phillips et al. 1994). Organelle composition and distribution is known to vary in somatic hybrids (Xu 1993; Xu et al. 1993), and this was examined in somatic hybrids between *S. sanctae-rosae* with *S. tuberosum* cv. Brodick.

Figure 2, an ethidium bromide-stained gel containing total DNA digested with *Bam*HI from these parents, shows unique DNA fragments. The prominent DNA fragments are typically derived from the chloroplast genome with other fragments of nuclear origin. After hybridisation to a biotin-labelled cpDNA, chemiluminescence detected several hybridised cpDNA sequences. Several other restriction enzymes were used to detect RFLP differences, however all cpDNA hybridised fragments were identical between the parents and hybrids. This clearly demonstrated the stability of the chloroplast genome in the somatic hybrids. To elucidate the chloroplast background in these hybrids we performed PCR analysis. Chloroplast-specific primers were screened to identify differences in PCR products between *S. sanctae-rosae* with *S. tuberosum* cv. Brodick (Fig. 3). Some 25 somatic hybrids were PCR-analysed, and most were shown to contain chloroplasts derived from *S. tuberosum* cv. Brodick with the exception of 1 somatic hybrid having the chloroplasts of *S. sanctae-rosae*. Organelle inheritance in plants is considered to be purely maternal (Reboud and Zeyl 1994), however in the production of somatic hybrids it is possible to generate biodiversity from the numerous cytoplasmic combinations (Kumar and Cocking 1987). In spite, of this wild species chloroplast component, it had little effect on the plants agronomic characters (plant height and tuber yield) compared to *S. tuberosum* cv. Brodick (results not shown), indicating cytoplasmic compatibility with the largely *S. tuberosum* cv. Brodick nuclear genome.

There is evidence for the genetic recombination and rearrangement of mtDNA in some somatic hybrids (Kanno et al. 1997). Indeed, early reports indicate there are several recombinational 'hot spots' where genetic exchange is likely to occur (Stern and Palmer 1984; Kemble et al. 1986). As the biogenesis of these organelles is similar, the most likely explanation for mtDNA to undergo genetic rearrangements is its size and its ability to exist in several genomic forms (Fauron et al. 1995). Plant chloroplast genomes are highly conserved in size and organisation, whereas mitochondrial genomes are variable in both of these parameters (Stern and Palmer 1984). The precise, regulatory mechanisms to trigger these recombinational events is uncertain but is often observed in tissue culture-derived plants (Kanno et al. 1997). This is clearly illustrated here, where RFLPs were recognised in several mt-DNA profiles after digestion with several different restriction enzymes (Fig. 4). Although these somatic hybrid mtDNA profiles were similar to *S. tuberosum* cv. Brodick, suggesting that this organelle component was inherited from *S. tuberosum* cv. Brodick subsequent to protoplast fusion, the missing mtDNA fragments are evidence of possible recombination events leading to genetic rearrangements. The full significance of these exchange events in the mitochrondrial genome and their effects on plant function and metabolic status remain to be elucidated, however, field studies over several years showed that several agronomic characters were unaffected in these somatic hybrids compared to *S. tuberosum* cv. Brodick.

Genetic modification via transformation is a directed, targeted procedure to introduce specific gene(s) into the plant's genome. This approach disrupts DNA sequences

during genomic integration and is likely to have consequences for gene expression (Birch 1997; Matzke and Matzke 1998; Kumpatla et al. 1998). Moreover, such events can affect higher order structures, where chromatin studies have shown alterations important to transgene transcriptional activity (van Blokland et al. 1997; Cheng et al. 1998). The significance of alterations in chromatin structure to ribosomal gene expression and transcriptional activity has been shown in yeast (Dammann et al. 1993, 1995). The modification of plant genomes via protoplast fusion is no exception to this process and is equally likelly, if not more so to undergo chromatin changes when nuclei fuse during heterokaryon formation.

In heterokaryons, parental chromosomes are likely to exchange genetic information, and inter-phase nuclei are certain to interact; these mechanisms of gene expression and regulation are fundamental in the formation of functional somatic hybrids. The endonuclease DNase I has been most widely used to characterise chromatin (Paul and Ferl 1988). As chromatin relaxes, DNA no longer becomes associated with histones and is vulnerable to enzymic digestion. Control of digestion can produce ladders of DNA, and the spacing of nucleosomes might change with the transcriptional state of the gene sequence (Felsenfeld et al. 1996). This has obvious implications for the ribosomal RNA genes and their expression in somatic hybrids, where actively transcribed areas show greater sensitivity to DNase I than inactive genes. Nuclei derived from *S. tuberosum* cv. Brodick were effectively degraded after DNase I treatment compared to those of *S. sanctaerosae* and the somatic hybrids which showed relative resistance to higher levels of DNase I (Fig. 5). Following hybridisation to an rDNA probe, resistance to DNase I digestion within the rDNA nucleosomal array was found in some somatic hybrids, whereas in others, the rDNA nucleosomal profiles were sensitive to higher DNase I concentrations relative to the parental profiles (Fig. 6). This differential sensitivity to DNase I treatment may be typical of tissue culture-derived plants, indicating profound alterations in chromatin structures and nucleosomal arrays. Indeed, these changes have been correlated with DNA methylation (van Blokland et al. 1997; Razin 1998) at the sites of transgene integration and acetylation of histone protein complexes (Bestor 1998).

Analysis of the parental plants and somatic hybrids may provide evidence for the mechanisms underlying these cellular/genetic functions. Changes in chromatin structure are often associated with alterations in gene expression, since DNA packaging into nucleosomes and higher order chromatin structures affect protein accessibility for transcriptional processes (Wellinger and Thoma 1997). The first level of chromosomal organisation is the condensation of DNA wound around histone octamers to form nucleosome arrays, while chromatin decondensation is normally associated with active gene expression (Workman and Kinston 1998). Transcriptional activity requires the disruption of nucleosomal structures and displacement of histones, in doing so, it generates nuclease-sensitive regions. The loss of nucleosomes from a transcribed gene has been demonstrated in ribosomal RNA genes (Dammann et al. 1993, 1995) and is evident in *S. tuberosum* cv. Brodick compared to the somatic hybrid plants. In these hybrids, the apparent resistance to DNase I activity is not solely restricted to the NOR region but is prevalent throughout the genome. This evidence supports the notion that some sequences within the ribosomal RNA gene tandem array are not expressed actively in these somatic hybrids. The suggested alteration in chromatin organisation (see Results) did not result in transient structures, as hybrid tubers could be maintained under field-grown conditions (see methods), indicating their stable somatic inheritance through several generations. Moreover, nucleosome structures and chromatin-folding processes are complex and have been associated with changing levels of DNA methylation (Razin 1998). All of these factors play a role in the formation of heterochromatin known for its transcriptional inactivity. Moreover, total RNA isolated from the somatic hybrids was hybridised to an rDNA probe, the results indicated that the levels of RNA (18 S + 25 S) derived from the ribosomal RNA genes were reduced in these somatic hybrids relative to *S. tuberosum* cv. Brodick (Harding and Millam 1999). Clearly, the simple transference of genetic information from a donor into a recipient cell, regardless of the modification process, is dependent on many interacting factors for the active gene expression essential for meeting the objectives of breeding programmes. The analysis of somatic hybrids, reported here shows the application of simple-sequence repeats in their identification, the use of PCR and Southern blotting techniques to examine the organelle composition of somatic hybrids and the need to examine nucleosomal structures important for transcriptional activity and gene expression.

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